

small amplitude (0.2%) length perturbations at 15 frequencies (1-250Hz). The experiments were performed at 15°C in the presence of 0.05-10 mM MgATP, 4 mM KH_2PO_4 , 4 mM K_2HPO_4 , 15 mM Na_2CP (creatine phosphate), 200 mM ionic strength with KAc (acetate), pCa 4.55, and pH 7.0. Two exponential processes B and C were resolved in tension transients. Their apparent rate constants ($2\pi b$ and $2\pi c$; $b < c$) increased ($[\text{MgATP}] = 0.05$ -1 mM) and saturated (≥ 1 mM) with an increase in the MgATP concentration. However, they were close together (1.4x) at $[\text{Pi}] \geq 4$ mM, hence they were combined to deduce the accurate estimate of the kinetic constants: their sum and product were analyzed as functions of $[\text{MgATP}]$. These analyses yielded $K_1 = 2.91 \pm 0.31 \text{ mM}^{-1}$, $k_2 = 288 \pm 36 \text{ s}^{-1}$, and $k_2 = 10 \pm 21 \text{ s}^{-1}$ ($\pm 95\%$ confidence limit, $N=13$). These results are consistent with the cross-bridge model: $\text{AM} + \text{ATP} \rightleftharpoons$ (step 1) $\text{AM} \cdot \text{ATP} \rightleftharpoons$ (step 2) $\text{A} + \text{M} \cdot \text{ATP}$. These kinetic constants compare to those observed in single fibers ($K_1 = 2.35 \pm 0.31 \text{ mM}^{-1}$, $k_2 = 243 \pm 22 \text{ s}^{-1}$, and $k_2 = 6 \pm 14 \text{ s}^{-1}$; $N=8$) under the same experimental conditions and analysis methods. These values are respectively not significantly different from those of myofibrils, indicating that the same kinetic constants can be deduced from myofibril and muscle fiber studies in terms of ATP binding and cross-bridge detachment steps. The fact that in myofibrils K_1 is 1.24x of that of fibers, may be explained by a small concentration gradient of ATP, ADP and/or Pi in single fibers.

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Muscle Cells from Lancelet: An Emerging Model for Sarcomere Dynamic Research

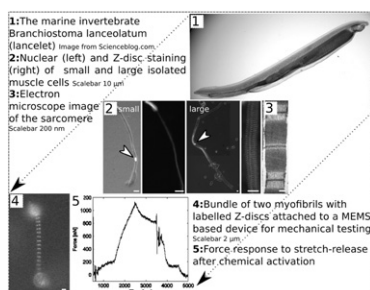
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The sarcomere and its networks are the mechanical driving mechanism of the animal kingdom. To overcome the fundamental limitations involved (network size and structural complexity) for the investigation of sarcomere dynamics in mammalian muscle, a uniquely small contractile system was introduced. The sarcomere network of lancelet is simply structured and exceptionally small. The aim of the study was to introduce cells from lancelet for the investigation of sarcomere function in an intact, self-regulated system.

Cells and myofibrils can be isolated and repeatedly activated. They can be adhered for force-length experiments and sarcomere tracking.

Mononuclear cells vary in length from 10^2 to $10^3 \mu\text{m}$ and 2-10 μm in diameter. The network is arranged in 1-20 myofibrils. The structure closely resembles the mammalian sarcomere having

a rest-length (L_0) of 2 μm . Force response of myofibrils to stretch-release resembles the behavior of mammalian myofibrils. Although the stretch response at $0.4 L_0 \cdot \text{s}^{-1}$ exceeds the isometric force by a factor of five. This is remarkably high indicating a distinct stabilization mechanism. In conclusion muscle from lancelet is a potential model for the study of interaction between sarcomere function and the behavior of the cell.



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Timing and Magnitude of Prestretch Strongly Affect Cardiac Muscle Tension Development

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Ectopic ventricular pacing leads to regional myocardial prestretch. In-vivo studies suggest that the timing of prestretch determines the effect on pump function and can lead to regional wall remodeling. Here we measure the direct effects of prestretch timing on the magnitude of tension development in isolated cardiac muscle. Prestretch strongly affected tension development but primarily during a brief interval of the twitch. The effects of prestretch were simulated using excised murine right ventricular papillary muscles. A high-speed computer-controlled motor was used to impose precisely timed stretches, while a force transducer measured force output and strain was monitored using a CCD camera and topical markers. The timing of the stretches greatly influenced tension production. A critical stretch timing interval was observed to begin 25 ms after stimulation and extending until 100 ms after stimulation, where stretches occurring in this interval exhibited statistically significant in-

creases in peak tension of as much as 500%. Muscle shortening occurring in the critical interval (from stretches initiated prior to stimulation) showed the opposite effect of stretching in the interval, with peak tension inhibited significantly by as much as 40%. A simple model showed that the varying impact of stretch timing could not be explained by time-varying elastance alone. Therefore, a more detailed and mechanistic myofilament model that included cooperative activation (Campbell et al 2010) was refined to include length dependence and strain-dependent cross-bridge kinetics. By comparing model results and experimental measurements, the strain history dependent mechanisms of these observations was evaluated. These evaluations have led us to the conclusion that even prestretches performed at slow physiologic velocities cause alterations in twitch kinetics that cannot be explained by time varying elastance.

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Effects of Ca^{2+} Concentration and MgADP on the Forces Produced After Stretch and Shortening of Skeletal Muscle Fibers

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The force produced by skeletal muscles increased after stretch and decreased after shortening, when compared to isometric contractions developed at corresponding lengths. The aim of this study was to compare the effects of Ca^{2+} -induced activation and MgADP-induced activation on the force produced after stretching or shortening. MgADP was used to assess if cross-bridges play a role in force changes, as it induces strong cross-bridge attachment to the thin filament. Two sets of experiments were performed with permeabilized fibres isolated from the psoas muscle: (i) fibres were activated at pCa2+ of 4.5 and 6.0, and (ii) fibres were activated at pCa2+ 4.5 before and after administration of 5 mM MgADP. In all experiments, fibres were initially activated to produce isometric contractions at nominal sarcomere lengths (SL) of 2.7, 2.85 and 2.6 μm . When fibres were activated at 2.6 and 2.85 μm (after full force development was obtained) they were stretched or shortened (5%SL at a speed of $1.0 \text{ SL} \cdot \text{s}^{-1}$), to reach a final SL of 2.7 μm . In all conditions, the force after stretch was higher than the isometric force at a corresponding length. The level of force enhancement was lower at pCa2+ 4.5 ($14.9 \pm 5.4\%$) than at pCa2+ 6.0 ($38.8 \pm 7.5\%$). Force did not decrease significantly after shortening at pCa 4.5, but it decreased by $17.2 \pm 5.6\%$ at pCa2+ 6.0. The levels of force enhancement and force depression were not changed when fibres were activated with MgADP, suggesting that Ca^{2+} concentration modulates the forces produced after length changes by mechanisms that are independent of cross-bridges activation of the thin filament.

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Contractile Properties of Myocardium Myofibrils Isolated from Mice with Heart-Specific Deletion of Arginyl-tRNA-Protein Transferase

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Protein arginylation is a cellular process catalyzed by arginyl-tRNA-protein transferase (Ate1). Mice with the *Ate1* knockout present high embryonic lethality and defects in cardiovascular development including myofibril disorganization, which may lead to heart disease. In this study we compared passive forces at sarcomere lengths (SL) between 1.8 and 2.6 μm , and contractile properties of myofibrils isolated from the myocardium muscle conditional knockout mice with heart-specific deletion of Ate1 with those from wild-type (WT) mice. The maximal isometric forces were lower in Ate1 KO myofibrils ($102.2 \pm 11.0 \text{ nN}/\mu\text{m}^2$) than in WT myofibrils ($151.3 \pm 11.7 \text{ nN}/\mu\text{m}^2$), which was accompanied by a downwards shift in the passive force-SL curve, suggesting that Ate1 KO myofibrils are weaker and less stiff. The rate of force development (Kact) was similar between groups (Ate1 KO: $3.1 \pm 0.4 \text{ sec}^{-1}$, WT: $3.3 \pm 0.5 \text{ sec}^{-1}$). The rate of force redevelopment (Ktr) following a shortening-stretch protocol was also similar between groups (Ate1 KO: $4.7 \pm 2.2 \text{ sec}^{-1}$, WT: $6.6 \pm 0.8 \text{ sec}^{-1}$), which suggests that changes in force are not associated with cross-bridge kinetics and thin filament activation. The rates of relaxation upon muscle deactivation (Krel) was similar among groups at most SL investigated, although there was a tendency for Ate1 KO myofibrils to become slower when measurements were made at short SL ($\sim 1.8 \mu\text{m}$). Overall, these results imply that arginylation is an important mechanism associated with force regulation in cardiac myofibrils, and it likely affects contractile proteins and passive structures within sarcomeres.